Smart Nanoparticles for Biological Imaging

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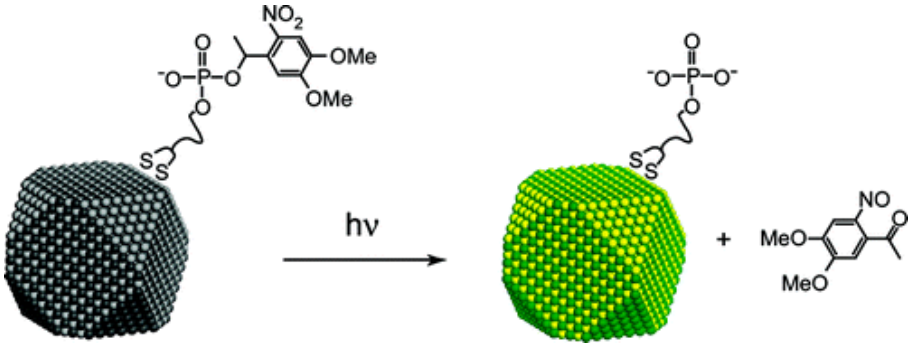
Scientific Thrust Area: Biological Nanostructures

Research Achievement: Certain nanoparticles possess unusual optical properties that are highly desirable in biology, which relies heavily on imaging probes and microscopy. We have recently developed photoactivatable nanoparticles—called *caged quantum dots*—that are non-luminescent under typical microscopic illumination but can be activated with stronger pulses of UV light. These nanoparticles are hybrids of hard and soft materials, and their unique optical properties arise from the interaction between a classic organic caging group and a semiconducting quantum dot (QD). We have demonstrated that these QDs can be photoactivated within live cells and have begun to examine the physical basis of the interaction between caging group and QD.



A second type of nanoparticle (developed in collaboration with Delia Milliron and James Schuck of the Molecular Foundry) with exceptional optical properties, the lanthanide-doped upconverting nanoparticle (UCNP), absorbs two photons in the nIR and emits one at shorter wavelengths in the visible or nIR. nIR excitation is exceptionally valuable for bioimaging: compared to the visible or UV, nIR radiation is less damaging to cells and scatters less, allowing deeper tissue penetration or even whole animal imaging. These particles emit "anti-Stokes" light, producing a higher-energy photon from multiple lower-energy photons, and because nothing in the cell emits anti-Stokes, there is no background autofluorescence with these nanoparticles. We have developed a synthesis of monodisperse UCNPs and a simple procedure for transferring them to water, and we have recorded the first single molecule images of UCNPs. We find that UCNPs do not blink, as QDs and organic probes do, and that they posses remarkable photostability, resisting photobleaching long after even QDs are extinguished.

Future Work: The two new particles described above will be further refined for work with cells. Caged QDs currently use UV-active caging groups as phototriggers, and while UV irradiation is commonly used to photoactivate proteins and organic probes, it is known to damage cells. We have synthesized a series of longer-wavelength caging

groups that can be photolyzed with the 488-nm source commonly found in imaging systems. These blue-light caging groups will also allow us to better understand the mechanism by which this class of compounds quench QDs so efficiently. We have previously found emission wavelength dependence of QD quenching; varying the caging group, along with computational efforts by Jeffery Neaton of the Molecular Foundry Theory Facility, should provide insight into the quenching mechanism.

Following our characterization of the single-molecule behaviors of lanthanide-doped upconverting nanoparticles, we have been pursuing several areas for improving their utility in cell imaging. We have developed a method of synthesizing smaller particles (< 10 nm) and are optimizing this synthesis using the WANDA robot in the Inorganic Facility. This smaller size regime is much more useful for bioimaging, with the larger particles more likely to perturb proteins being studied in tracking experiments. We are also pursuing other lanthanide combinations to vary the excitation wavelengths to avoid nIR water absorbances that may interfere with live cell imaging. The photostability and lack of blinking of UCNPs makes them ideal for tracking the motions of membrane proteins such as G-protein coupled receptors, which are critical in a variety of cellular functions. We have coated UCNPs with antibody-binding proteins and will pair these with antibodies to cellular receptors for long-term tracking experiments.

Publications:

C. Ajo-Franklin, G. Han, T. Mokari, B.E. Cohen. Caged Quantum Dots. *J. Am. Chem. Soc.* 130, 15811-15813 (2008).

S. Wu, G. Han, D.J. Milliron, S. Aloni, V. Altoe, D.V. Talapin, B.E. Cohen, P.J. Schuck. Non-blinking and photostable upconverted luminescence from single lanthanide-doped nanocrystals. *Proc. Nat. Acad. Sci.*, in press.